Introduction

Submerged Aquatic Vegetation (SAV) is a group of rooted, vascular macroscopic aquatic plants found throughout the shallow tidal and non-tidal waters of the Chesapeake Bay and its tributaries. SAV serves many essential functions in maintaining a healthy Chesapeake Bay ecosystem including: producing oxygen, providing food for a variety of animals and waterfowl, providing shelter and nursery habitat for juvenile fish and crabs, and reducing pollution and improving water quality by absorbing nutrients and trapping sediments. Studies and historical documents indicate that until the early 1970's, SAV had been continuously present in many regions of the Chesapeake Bay, and the Patuxent River specifically, for the past 1200 years. The Patuxent River estuary, like many other temperate estuaries, exhibited dramatic declines in the abundance of SAV during the later half of the 20th century coincident with increasing population density and nutrient loading within the watershed (Den Hartog and Polderman 1975; Orth and Moore 1983; Cambridge and McComb 1984; Orth et al. 1994). A study utilizing pollen dated sediment cores found that at three locations on the Patuxent estuary, SAV was found continuously from approximately 1200 AD to the early 1970's- at which time seeds disappeared from the sediment record (Brush and Hilgartner 2000).

In the late 1960s and 1970s, increases in nutrient and sediment inputs from development of the surrounding watershed (Kemp et al. 1983) contributed to a sharp decline in SAV populations baywide (Orth and Moore 1983). SAV populations began to rebound in

1984 increasing from 38,000 to 90,000 acres in 2002. However, a dramatic baywide decrease was seen in 2003 when SAV populations declined to less than 65,000 acres.

The Chesapeake Bay Program and SAV

Because of its role in providing habitat, retaining sediment and improving water quality, the restoration of SAV has become an important component of the U.S. Environmental Protection Agency's (EPA) Chesapeake Bay Program (CBP) goals. Over the past 20 years, the CBP has committed significant resources to determining the causes for SAV decline and to identify the best course of action for protecting and restoring natural populations.

In 2003, the CBP adopted a goal seeking to increase SAV acreage in the Chesapeake Bay to 185,000 acres by 2010. The CBP simultaneously created the "*Strategy to Accelerate the Protection and Restoration of Submerged Aquatic Vegetation in the Chesapeake Bay*". The Strategy, the result of more than a yearlong effort among Chesapeake Bay SAV researchers and managers, identified the major actions necessary to successfully increase SAV populations in the Bay. These actions fall into four major categories:

1. Improve water clarity sufficient for supporting healthy SAV populations
2. Protect existing beds from impacts by anthropogenic sources and exotic species
3. Plant or reseed 1,000 acres in strategic locations by December of 2008
4. Conduct applied research and public education / outreach on the benefits of healthy SAV beds.

Large scale restoration efforts were deemed necessary to accomplish Action #3 outlined in the Strategy: to plant or reseed 1,000 acres in strategic locations by December of 2008, and ultimately to meet the CBP goal of 185,000 acres, baywide, by the year 2010.

Patuxent River

As of fall 2005, the Patuxent River is one of only a few sites in Maryland and Virginia that has undergone the two-year site selection process (test plantings and water quality monitoring) outlined as a requirement of the strategy for large scale restoration locations. The Patuxent is the largest river completely in the State of Maryland, draining 932 square miles of land from portions of St. Mary's, Calvert, Charles, Anne Arundel, Prince George's, Howard, and Montgomery Counties (Patuxent River Commission Staff 2003). It is one of the most intensively monitored and modeled rivers of its size in the world, and therefore, serves as an important proving ground for many of the CBP initiatives (Maryland Department of Natural Resources 2005).

Prior to the decline of SAV beds in Chesapeake Bay between the 1960's and 1970's, the Patuxent River supported diverse populations of SAV including *Zannichellia palistris*, *Ruppia maritima*, *Potamogeton perfoliatus* and *Zostera marina* (Brush and Davis 1984). Both stratigraphic records and groundtruthing evidence suggests the presence of *Z. marina*, eelgrass, historically throughout the mesohaline portion of the Patuxent River. The disappearance of these species coincided with the degradation of water quality across the Bay region during this period. Like all other tributaries of the Chesapeake Bay, the Patuxent River has been impacted by nutrient pollution. Excess nitrogen and phosphorus stimulate algae growth. The combination of excess algal growth and suspended sediments can increase light attenuation in the water column and inhibit the growth of SAV. Managers have set forth nutrient reduction goals and have addressed sediment pollution to promote the resurgence of submerged aquatic vegetation and improve habitats (Patuxent River Commission Staff 2003). Nitrogen loads in the Patuxent River were reduced from 5.02 to 4.07 million pounds a year (19%) and phosphorus was reduced from 0.51 to 0.27 million pounds a year (47%) from 1985-2003 (Patuxent River Commission Staff 2003).

A resurgence of SAV in the tidal freshwater reach and middle portions of the Patuxent River since 1993 has been attributed to significant reductions in pollutant loads and resulting improvements in water clarity (Naylor and Kazyak 1995). The 2004 aerial survey recorded 220 acres of SAV in the tidal fresh portion, 4,340 percent of the goal for this portion of the river, and 106 acres in the middle portion, or 156 percent of the goal for that area (Maryland Department of Natural Resources 2005). However, SAV populations remain sparse in the lower mesohaline region of the Patuxent. Only 142 acres were mapped in 2004, far below the 1,325-acre goal for this portion of the river (Maryland Department of Natural Resources 2005) (Figure 1).

Recent analysis by Stankelis et al. (2003) has suggested that the mesohaline portion of the Patuxent River may be inappropriate for SAV restoration based on the reduction of light available to SAV. In the mid-mesohaline portion (just below Broomes Island), continued poor water quality (low secchi depth measurements) was suspected to be responsible for losses and lack of revegetation of SAV. In the lower mesohaline region (near Solomons Island), secchi depth measurements indicated water quality conditions suitable to sustain SAV growth. However, significant light attenuation due to high epiphyte loading was thought to cause the loss of SAV from this area.

Epiphyte test strips deployed in the lower mesohaline region (Drum Point to Solomons Island) have shown elevated light attenuation rates, between 30-70 % of surface light, high enough to cause a loss of plants due to leaf-surface light attenuation (Stankelis 2003). Despite this, habitat assessments and initial test plantings (small eelgrass plots,1-3 m^2) over the past three years have provided evidence that water quality in this same region (Broomes Island to Drum Point) of the river could support eelgrass beds (Maryland Department of Natural Resources and Dr. Walter Boynton, University of Maryland, unpublished data). Water quality data from 1985-2003 analyzed by the Chesapeake Bay Program Tidal Monitoring and Analysis Workgroup indicated that in the lower Patuxent River, total suspended solids, nitrogen levels, and percent light at leaf (PLL, the amount of ambient surface light required at the leaf surface to support growth) all pass the SAV habitat requirements, while light attenuation and chlorophyll levels remained below satisfactory levels (Maryland Department of Natural Resources 2005) (Figure 2). In addition to epiphyte loading, predation, specifically damage by mute swans (*Cygnus olor*) and cownose rays (*Rhinoptera bonasus*), may hinder the success of SAV (Orth 1975). Furthermore, the nature of the test plots- small, low-density plantings, has made them particularly susceptible to both physical damage and epiphyte loading.

The combination of documented historical eelgrass coverage, water quality meeting the SAV habitat requirements according to the SAV targeting system (Parham and Karrh 1998), and the vast water quality dataset for the Patuxent River make this river a prime candidate for large scale eelgrass restoration.

Eelgrass in Restoration

Eelgrass was widely distributed in parts of the Patuxent River and Chesapeake Bay until the late 1960's (Brush and Davis 1984; Orth et al. 2003). Eelgrass was also identified in the SAV Strategy as one of two species with great potential for large-scale restoration in the Chesapeake Bay. This perennial seagrass is capable of both vegetative and sexual reproduction. Reproductive structures form when water temperatures reach 10-15^{o}C and seed production begins when average temperatures reach 15-20^{o}C (Granger et al. 2002), typically late May through early June in the Chesapeake Bay (Silberhorn et al. 1983). Seeds can be released from reproductive shoots in close proximity to the parent bed or shoots with mature seeds still intact may also break free from the plant and be exported from the bed (Orth et al. 1994), serving as a vehicle for long distance dispersal (McCroy 1968). Upon release from reproductive shoots, mature, negatively buoyant seeds fall to the bottom of the water column or are transported from the bed (Orth et al. 1994). Eelgrass seed germination in the Chesapeake Bay appears to be dependent upon water temperature, burial of seeds, and oxygen concentrations (Orth and Moore 1983; Moore et al. 1993), and typically begins in mid-October when water temperatures drop below 15 ^{o}C (Moore et al. 1993).

Early restoration efforts involved transplanting adult eelgrass plants from healthy source beds to restoration locations. Averaging 37,000 dollars per acre (Fonseca et al. 1998) plus additional cost for monitoring, this transplanting method is expensive and labor intensive, and often resulted in minimal success. While transplanting is still utilized as a restoration method, there has been increasing interest in using eelgrass seeds for restoration. Seed broadcasting appears to be a more efficient and cost effective restoration technique (Orth et al. 2000) with the added benefit of having less impact on donor beds.

To collect seeds for use in restoration efforts, reproductive shoots are harvested from healthy donor beds when mature (Granger et al. 2002). Seeds are held in large tanks under ambient conditions until mature, separated from reproductive spathes, and stored for up to 3 months (Granger et al. 2002). Seed dispersal (Orth et al. 1994) typically takes place from mid-August to mid-October before water temperatures drop below 15°C (Orth and Moore 1983; Moore et al. 1993).

Recently, an alternative to traditional seed collection and storage has been developed. Using techniques similar to the buoy-deployed seeding system (BuDSS) developed by Pickerell et al. (2003; 2005), freshly harvested reproductive shoots are placed in mesh bags immediately after harvest, moved to the restoration location, attached to anchored buoys, and deployed in the area to be restored. The mesh bags remain suspended at the top of the water column, allowing the seeds to develop, mature, and drop out over a period of weeks. This mimics the floating and rafting of reproductive shoots during natural seeding events (Pickerel et al. 2003; 2005). This method eliminates the need to store seeds, reducing the number of seeds lost to processing, and decreases the expense and labor requirements associated with seed transport, processing, and storage. Initial restoration efforts using the BuDSS in the Peconic Estuary, NY yielded up to 4% recruitment (Pickerell et al. 2003; 2005).

Transplanted or seeded SAV beds have the potential to thrive and provide benefits similar to naturally occurring beds (Fonseca et al. 1994). There are several regions within Chesapeake Bay in which habitat conditions have shown significant improvement since long term monitoring began in 1985 and are now suitable for SAV recolonization (Maryland Department of Natural Resources). However, many of these regions remain unvegetated due to a lack of SAV seed or propagule sources. By identifying these areas and strategically seeding them, it is hoped that significant numbers of plants will germinate and grow to establish dense, self-protecting beds. The combination of self-protection and reproduction within these beds should generate seeds that may accelerate natural revegetation of areas adjacent to the restored beds (Orth et al. 2003).

The MD-DNR has developed this project to conduct large scale eelgrass restoration at select locations on the Patuxent River, MD. This project will consider previous restoration efforts while investigating new technologies in order to meet project goals and maximize the area that is restored. The following objectives will be met by the conclusion of this project:

1. Identify sites for restoration based on application of GIS based targeting models, recent and on-going test plantings, and intensive habitat assessments.
2. Conduct large-scale seeding of eelgrass at each site over a three-year period as called for in the Strategy.
3. Evaluate associated factors that may influence success of the project such as seeding density, water quality, epiphytic growth, and predation.
4. Produce a final, technical analysis documenting degree of revegetation of each site and evaluating the role of associated factors.

To address these objectives, this project compared the seedling success, as well as the budget requirements, of two seed dispersal methods while simultaneously investigating associated factors that may be contributing to low germination rates and seedling success in the Patuxent River. The first two years of this project were devoted to site selection, which involved applying existing habitat information to identify general areas suitable for restoration and test plantings at specific sites. Large scale broadcast seeding and seed bag deployments were utilized and their relative successes and associated costs were compared. This report presents results and conclusions from the site selection process, the first 2 years of seeding (2003 and 2004), and the relative effects of associated factors on the success of restoration efforts.